

**Comparison of inclusion of skimmed milk and edible insects on the nutritional, sensory and microbial qualities of enriched high energy rice biscuits**

---

**ABSTRACT**

**Aims:**The growing world population, carbon foot prints of rearing conventional protein sources, issues of protein malnutrition (particularly in developing nations) and the urgent needs of protein-rich food relief materials in crisis situations may necessitate the utilisation of underutilised sustainable, nutritious protein sources. The study was conducted to investigate the possibility of using indigenous food crops and substituting milk powder with edible insects' powder as a sustainable alternative protein source in high energy rice biscuits

**Study design:**Biscuits were produced following the standard USAID recipe for HEB and comparisons were made with the insect biscuits and STD

**Place and Duration of Study:** Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria, between January 2018 and August 2018.

**Methodology:**The high energy biscuits was evaluated for proximate composition, total sugars using Anthrone reagent, micronutrients (vitamins and minerals), physical properties such as spread ratio, weight, width etc. Microbial and sensory quality of the biscuits were also determined using standard methods.

**Results:**From the findings of the study, rice biscuits with silkworm pupae had outstanding protein (12.76 g/100 g), fat (15.78 g/100 g) and energy (451.38 Kcal/100 g) contents while biscuit with migratory locust powder had slightly higher total ash and CHO. The spread ratios of the biscuits were within acceptable range. In terms of mineral composition, all the insect biscuits were significantly superior to the milk biscuit except for calcium. However, all the rice HEB exceeded the minimum USAID specifications for nutritive elements. The insect biscuits had more than quadruple the recommended pro-vitamin A content (1200.67 and 2390.05 µg/100 g) and impressive amounts of vitamin C (94.19 and 52.39 mg/100 g). The microbial assessment revealed HEB with remarkable quality signifying wholesome raw materials and hygienic sample preparation. All samples received good sensorial ranking, but HEB with migratory locust was preferred.

**Conclusion:**The results demonstrated that edible insects could serve as alternative protein source in the production of a nutritious high energy biscuit with good organoleptic and microbiological qualities.

*Keywords:*Mulberry silkworm pupae, migratory locust, alternative protein, High-energy biscuits, nutritional composition, rice

## **1. INTRODUCTION**

According to research, the global consumption of protein will nearly triple before 2050 because of the expanding world population, speedy urbanization, depletion of land resources, global warming, environmental issues triggered by traditional cattle rearing, shift in consumption patterns amongst others [1]. In order to meet this developing demand and to go toward sustainable nutrition, there is urgent need to strengthen new and

neglected sources of dietary proteins as a complement or alternative to the existing conventional sources of proteins such as beef, fish, egg, milk [2].

Among the new and neglected sources of proteins, edible insects are one of the most booming resources and one of the largest organic groups in earth organism [3]. With its vast resources, speed of reproduction, lower feed consumption, low greenhouse gas emissions, less water utilization, and incredible amino acid, fatty acid and micronutrient profiles, edible insects are an important food resource with its enormous viability for improvement and utilisation for human food products [1]. More than 1900 species of insects have been described in literature as edible, and majority of them are in tropical nations where protein malnutrition is profound [3]. Some of the commonly consumed insects in no particular order are termites, crickets, grasshoppers, palm weevil larvae, silkworm pupae, and migratory locust. Ordinarily, insects are now not used as emergency food to ward off starvation, but are covered as an everyday part of the eating regimen at some stage in the year or when seasonally available in Africa, Asia etc. This seasonal level of insect consumption is gradually reducing due to westernization and lack of introduction of edible resources to the upcoming generation while some people with some level of exposure are now expressing disgust and neophobia particularly to edible insects in its visible whole forms. Incorporation of edible insects into products in concealed forms such as powder, batter, paste had been reported to enhance the nutritional quality of food products and acceptability of edible insects [4,5,6,7,8,9,10,11].

Ready to eat snacks such as biscuits have been advocated as a means to enlarge the dietary needs of consumers by incorporating nutrients such as proteins, fibres and phytochemicals which have health advantages [12]. High energy biscuit is one of such biscuits that has been packed with macronutrients and micronutrients to meet the nutritional demands of nutritional challenged people and/or people in emergencies. However, in cases of emergencies, high energy biscuits (made of wheat, skimmed milk, vitamin and mineral premix) are usually sourced from distant countries increasing cost and time of interventions. To facilitate quick response, developing an alternative to the HEB using locally readily available and nutritionally comparable resources such as local rice flour, edible insects, orange fleshed sweet potato and moringa leaves could be an advantageous approach.

Although, there are growing interest to incorporate insect derived ingredients into food products, more researches are still required to establish its use as a protein resource in foods for the people with nutritional challenges. Hence, this study was intended to assess the nutritional, sensory and microbiological qualities of rice-based high energy biscuits with mulberry silkworm pupae or migratory locust powder as an alternative to skimmed milk powder.

## **2. MATERIAL AND METHODS**

### **2.1 Sources of materials**

Edible insects; mulberry silkworm pupae was procured from Ondo state Agri-business Empowerment Centre, Akure. Migratory locust was purchased from Katsina State, Nigeria. Sweet Potato (orange fleshed variety) was sourced from Institute of Root and Tuber Crops, Umudike, Abia State, Nigeria. Moringa leaves were sourced from a residential garden around FUTA. Local rice, icing sugar, shortening and other ingredients were obtained from a local market in Akure, Ondo State.

### **2.2 Sample preparation**

#### **2.2.1 Production of edible insects' powder**

Freshly harvested silkworm pupa from the sericulture unit of Ondo state Agri-business Empowerment Centre were muted in an oven for 1 h at 93<sup>0</sup> C. The stifled silkworm pupae were removed from their cocoon by cutting and further heated in a Gen. Lab. oven for 8 hours at 40<sup>0</sup> C to facilitate drying and milling. The insects were milled into powder using electric blender (Binatone, Model No: 51-777) and stored airtight. Uncooked dry locusts were cleaned and sorted removing the frass, legs and wings. The remaining wholesome parts were milled (ATLAS exclusive ALZICO LTD. Type: YL 90L- 4) to powder, sieved and kept airtight [6].

### **2.2.2 Production of orange fleshed sweet potato to flour**

The OFSP were processed immediately on its arrival at the laboratory. They were peeled, washed with potable water, sliced into thin slices to reduce the surface area for fast drying and soaked in water containing 1% NaCl, 0.5% citric acid and 1% sodium metabisulphite for 30mins to prevent colour changes during drying. The soaked slices were drained and dried in oven (Gen Lab. Oven) at 55<sup>0</sup> C for 7 h, finely milled into flour using disc attrition mill (ATLAS exclusive ALZICO LTD), sieved and stored airtight.

### **2.2.3 Production of rice flour and moringa leaves powder**

Firstly, the rice was sorted and cleaned to remove unwanted foreign materials, followed by milling using disc attrition mill (ATLAS exclusive ALZICO LTD. Type: YL 90L- 4) and sieved. Healthy moringa leaves were wet cleaned with potable water, initially air-dried and subsequently oven-dried for a period of eight hours at 55 °C. The dried leaves were then milled employing the use of a Binatone electric blender (Model No: 51-777), sieved and kept away from atmospheric air.

### **2.2.4 Rice-based HEB formulation and processing**

The high energy rice biscuit was processed following the formulation as expressed in Table 1. Hot liquid shortening and icing sugar were taken and creamed to a uniform consistency. The composite flours (rice and OFSP flours), edible insect flour or skimmed milk, baking powder, moringa leaf flour and guar gum and 200 mL of water were added to creamed mixture and mixed for 10 min. The resultant dough was rolled out into thin sheet of uniform thickness and was cut into desired shape using a stainless circular biscuit cutter of 1½ inches/3.8 cm (PANNIUZHE. Model: 12PCS). The cut pieces of the enriched rice biscuits were placed on a tray and transferred into pre-heated convective baking gas oven (Scan frost. Model: PRG96G2G) at 180 °C for 15 min until baked. The well baked high energy rice biscuits were removed from the oven, cooled to room temperature, packed and stored at room temperature for further analyses.

**Table 1. Formulations of the High Energy Rice Biscuits**

Ingredients	(%)	(g)
Rice flour	40	120
<sup>1</sup> SWP or <sup>2</sup> MLP or <sup>3</sup> MP	15	45
Icing sugar	15	45
Refined palm olein oil	15	45
OFSP flour	12	36
Moringa leaf powder	1.6	4.8
Baking powder	1.4	4.2
Guar gum		3

SWP: Silkworm pupae powder; MLP: Migratory locust powder; MP: Skimmed milk powder  
OFSP: Orange-fleshed sweet potato.

## 2.3 Methods

### 2.3.1 Proximate analyses

Proximate composition was carried out on the high energy rice biscuits according to the method of AOAC [13]. Moisture and protein contents were determined using air oven and Kjeldahl method, respectively. Acid and alkali digestion of the samples followed by drying and ashing were used in estimating the crude fibre content of the samples. Samples were extracted using hexane solvent to determine the fat content. Percentage ash was determined by igniting the samples in a muffle furnace at 550 °C for six hours. Carbohydrate was obtained by difference and energy values estimated according to Atwater system. All analyses were carried out in triplicate.

$$\text{Energy} = [(\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)]$$

### 2.3.2 Mineral composition

The nutritive elements (calcium, iron, magnesium, and zinc) of the rice biscuits were determined following AOAC [14] method. In brief, one gram of the ground biscuits was ashed for two hours at 500 °C. The resultant ash was moistened with ten drops of water and 3 – 4 mL of nitric acid. Excess nitric acid was evaporated at 100 - 120 °C on a hot plate. The obtained ash was dissolved in 10 mL hydrochloric acid, and made up to 50 mL with distilled water. The concentration of each element was determined using an ICP (Inductively-Coupled Plasma) Optical Emission Spectrometer (ICP-OES – Horiba, France SAS). Potassium were determined using flame emission photometer (Sherwood Flame Photometer, Cambridge, UK) with NaCl and KCl as the standards. Phosphorus were determined using Vanado-molybdate method as described by Jacob *et al.* [15].

### 2.3.3 Pro-vitamin A assay

The rice biscuit was analysed for pro-vitamin A according to the procedure of De Carvalho *et al.* [16] with slight modifications. Briefly, one gram of the defatted sample was homogenized in 30 mL absolute alcohol and 3 mL of 5% potassium hydroxide. The mixture was boiled gently under reflux for 30 min in a stream, cooled and washed into a separator with 30 mL distilled water. Vitamin A was extracted from the mixture by shaking three times with fifty mL ether. The lower part of the separated mixture was discarded and the extract was washed four times with 50 ml water. Evaporation of the extract to five mL was done, followed by flushing with nitrogen. The obtained residue was then dissolved and made up to 25 ml with petroleum ether, filtered through 45 µm. The absorbance was read at 450 nm (Beckman Spectrophotometer – Model 35 DB).

$$\text{Pro - vitamin A } (\mu\text{g/g}) = \frac{V \times 10000 \times A_{450}}{W \times A_{1\text{cm}}^{1\%}}$$

V = total extract volume;  $A_{450}$  Absorbance at 450 nm; W = weight of the sample; and  $A_{1\text{cm}}^{1\%} = 2592$  ( $\beta$ -carotene extinction coefficient in petroleum ether)

### **2.3.4 Vitamin C assay**

Vitamin C was determined spectrophotometrically using the method described by Kapur *et al.* [17]. Fifteen grams of the sample was homogenized with one percent w/v metaphosphoric-acetic acids solution (25 mL), thoroughly shaken and made up to 50 mL. The resulting solution was filtered and centrifuged at 4000 rev per min. After, the centrifugation, the supernatant was kept as sample aliquot. Four millilitres of the sample aliquot was mixed with 0.23 mL of 3% bromine water to oxidize ascorbic acid of the sample into dehydroascorbic acid. Thiurea (0.13 mL of 10%) was used to remove excess bromine. Subsequently, one mL of 2, 4-DNPH reactant was added to form osazone. The sample, blank and standard solutions were kept at 37 °C for three hours, cooled in ice bath and treated with five mL of 85% chilled  $\text{H}_2\text{SO}_4$ , with continual stirring. The absorbance of the red coloured solution was read at 521 nm. Ascorbic acid content of the sample in mg/100 g was extrapolated from a calibration curve.

### **2.3.5 Total sugar content**

Anthrone Reagent was used for the determination of the total sugar content of the enriched rice biscuit. One gram of the sample was hydrolysed with 5 mL of 2.5 N hydrochloric acid in a water bath for a period of three hours. It was allowed to cool and neutralized with  $\text{Na}_2\text{CO}_3$  until effervescence ceased. The resulting mixture was made up to 100 mL and centrifugation was carried out for 5 min at 6000 rev per min. Anthrone reagent (4 mL) was added to the aliquot supernatant (1 mL), boiled in a water bath for 8 min, cooled and the resultant green to dark green colour was measured using a spectrometer (HITACHI spectrophotometer. Model: EA6000VX) at 630 nm. The absorbance value was compared with the standard curve of total carbohydrate and total sugar (glucose) was estimated as follows:

$$\text{Total sugar (mg/100 g)} = \frac{\text{Amount of carbohydrate} / 1 \text{ g} \times \text{volume of test sample}}{100}$$

### **2.3.6 Assessment of microbial quality**

Total viable aerobic mesophilic bacteria (TVMB) were determined using ten-fold serial dilution [18]. To two grams of the sample, 10 mL of the saline water was added and thoroughly shaken to dislodge microbes. Subsequently, ten-fold dilutions were carried out by adding one mL of the previous dilution to 9 ml of fresh sterile diluents. Finally, using a syringe, 1 ml of an appropriate dilution was transferred into a petri dish. Nutrient agar was poured into respective petri dishes for each sample, allowed to cool, solidify and incubated at 30 °C for a day. After the incubation period, enumeration of the noticeable growths were carried out. The presence of other microbes such as *Bacillus cereus*, Coliforms, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were determined likewise but with appropriate media, Nutrient agar, MacConkey agar, Eosin methylene blue, *Salmonella-Shigella* agar and Mannitol salt agar, respectively and incubated at 30 °C for 24 h. Similar to TVMB, yeast and mould counts were determined using Potato dextrose agar and incubated at 25 °C for 72 h.

### **2.3.7 Sensorial assessment**

Sensory attributes of the high energy rice biscuits were conducted using twenty untrained panelists. The objective of the study was communicated to respondents according to the ethical requirement of the university. The rice biscuits were evaluated for appearance, aroma, colour, crispiness, taste and overall acceptability on a nine-hedonic scale, in which 1 stands for extremely disliked and 9 extremely liked. Samples were presented in a

randomized order in small, white SMART plastic plates for each of the samples. Evaluation was carried in a well-lit environment without distractions.

## 2.4 Data analyses

Statistical analysis was carried out using Microsoft Excel (version 2013). Data were expressed as the mean  $\pm$  standard deviation of three replicates. One-way analysis of variance (ANOVA) was performed and means were compared by Duncan Multiple Range Test at 95% confidence level.

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate composition of high energy biscuit

The level of moisture in the three high energy rice biscuits vary significantly and were considerably lower than the USAID [19] maximum requirement for moisture (Table 2). This implies the rice HEB will be safe against moisture related deterioration. The insect-biscuits and the skimmed milk biscuit contain high amount of protein when compared with the minimum standard requirement of 10%. Protein content ranged from 11.33 to 12.76% with HEB containing silkworm pupae having the highest significant protein content. Edible insects are notably for its high protein and can serve as a potential alternative to traditional protein sources. The biscuits however differs considerably in its fat content with the control (HEB with skimmed milk) falling about 26% short of the minimum required fat content. The insect enriched biscuits however exceeded the USAID [19] minimum fat requirement by approximately 25%. A similar observation was reported for muffins and cookies substituted with caterpillar, meal worm and grasshopper powders[20,21,22]. After protein, fat is another principal component of insects [23]. Insects' fats are predominantly unsaturated fatty acids consisting of monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids such as omega-3 fatty acids are indispensable for normal cellular functioning of the human body and hence must be supplied by the diet. Insect HEBs were slightly lower in ash when compared with milk HEB. Ash content is a reflection of composition of minerals of the products. Higher ash levels of the skimmed milk HEB may be attributed to use of commercial fortified skimmed milk in the formulation of the milk HEB. The crude fibre and total sugar contents of the HEBs were within the specified 2.3% maximum and 10 g minimum, respectively. A higher crude fibre and ash contents were reported by Akande *et al.* [6] for high energy maize biscuits. The carbohydrate and energy contents of the rice biscuits followed the same trend. The milk biscuit had lower and higher significant energy and carbohydrate contents, respectively. The high nutritional profile of the edible insect enriched biscuits makes insect food products a promising dietary intervention for nutritionally challenged people particularly protein-energy related malnutrition which are prevalent in underdeveloped and developing nations.

**Table 2. Nutritional composition of the high energy rice biscuits**

Parameters	HEB_SWP	HEB_MLP	HEB_MP	STD
Moisture	2.64 $\pm$ 0.12 <sup>a</sup>	2.23 $\pm$ 0.11 <sup>b</sup>	1.91 $\pm$ 0.11 <sup>c</sup>	4.5% max.
Crude protein	12.76 $\pm$ 0.06 <sup>a</sup>	11.33 $\pm$ 0.09 <sup>c</sup>	12.46 $\pm$ 0.07 <sup>b</sup>	10% min.
Total fat	15.78 $\pm$ 0.71 <sup>a</sup>	15.43 $\pm$ 1.04 <sup>a</sup>	8.90 $\pm$ 0.70 <sup>b</sup>	12% min.
Crude fibre	2.18 $\pm$ 0.20 <sup>ab</sup>	1.98 $\pm$ 0.20 <sup>b</sup>	2.08 $\pm$ 0.10 <sup>ab</sup>	2.3% max.
Total ash	2.07 $\pm$ 0.20 <sup>b</sup>	2.06 $\pm$ 0.19 <sup>b</sup>	2.71 $\pm$ 0.19 <sup>a</sup>	3.5% min.
Carbohydrate	64.58 $\pm$ 0.86 <sup>c</sup>	66.97 $\pm$ 1.01 <sup>b</sup>	71.94 $\pm$ 0.84 <sup>a</sup>	-
Total sugars	24.36 $\pm$ 0.03 <sup>ab</sup>	25.05 $\pm$ 0.09 <sup>a</sup>	24.67 $\pm$ 0.09 <sup>ab</sup>	10 g min.
Energy	451.38 $\pm$ 0.94 <sup>a</sup>	452.07 $\pm$ 2.16 <sup>c</sup>	417.76 $\pm$ 1.61 <sup>b</sup>	462 Kcal min.

All values are mean  $\pm$ SD of three replicates. Values with different alphabet across a row are significantly different ( $P= 0.05$ )

HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder; STD: USAID [19] standards for high energy biscuits

### 3.2 Physical parameters of high energy rice biscuits

All the high energy rice biscuits were not significantly different in terms of their weight, width and spread ratio (Table 3). Spread ratio had been attributed to water absorption capacity of the flours leading to aggregation and competition of ingredients for available water during baking [24]. Variation in the thickness of the biscuits could be attributed to the type of protein incorporated as the protein source.

**Table 3. Physical parameters of the enriched HEBs**

Sample	Thickness (mm)	Width (mm)	Weight (g)	Spread ratio
HEB_SWP	0.53 $\pm$ 0.03 <sup>b</sup>	4.53 $\pm$ 0.03 <sup>a</sup>	7.57 $\pm$ 0.68 <sup>a</sup>	8.57 $\pm$ 0.53 <sup>a</sup>
HEB_MLP	0.73 $\pm$ 0.09 <sup>a</sup>	4.63 $\pm$ 0.09 <sup>a</sup>	6.91 $\pm$ 0.67 <sup>a</sup>	6.51 $\pm$ 0.79 <sup>a</sup>
HEB_MP	0.50 $\pm$ 0.01 <sup>b</sup>	4.43 $\pm$ 0.07 <sup>a</sup>	8.85 $\pm$ 0.75 <sup>a</sup>	8.87 $\pm$ 0.13 <sup>a</sup>

All values are mean  $\pm$ SD of three replicates. Values with different alphabet across column are significantly different ( $P = 0.05$ ),HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder

### 3.3. Vitamin and Mineral composition of the high energy rice biscuits

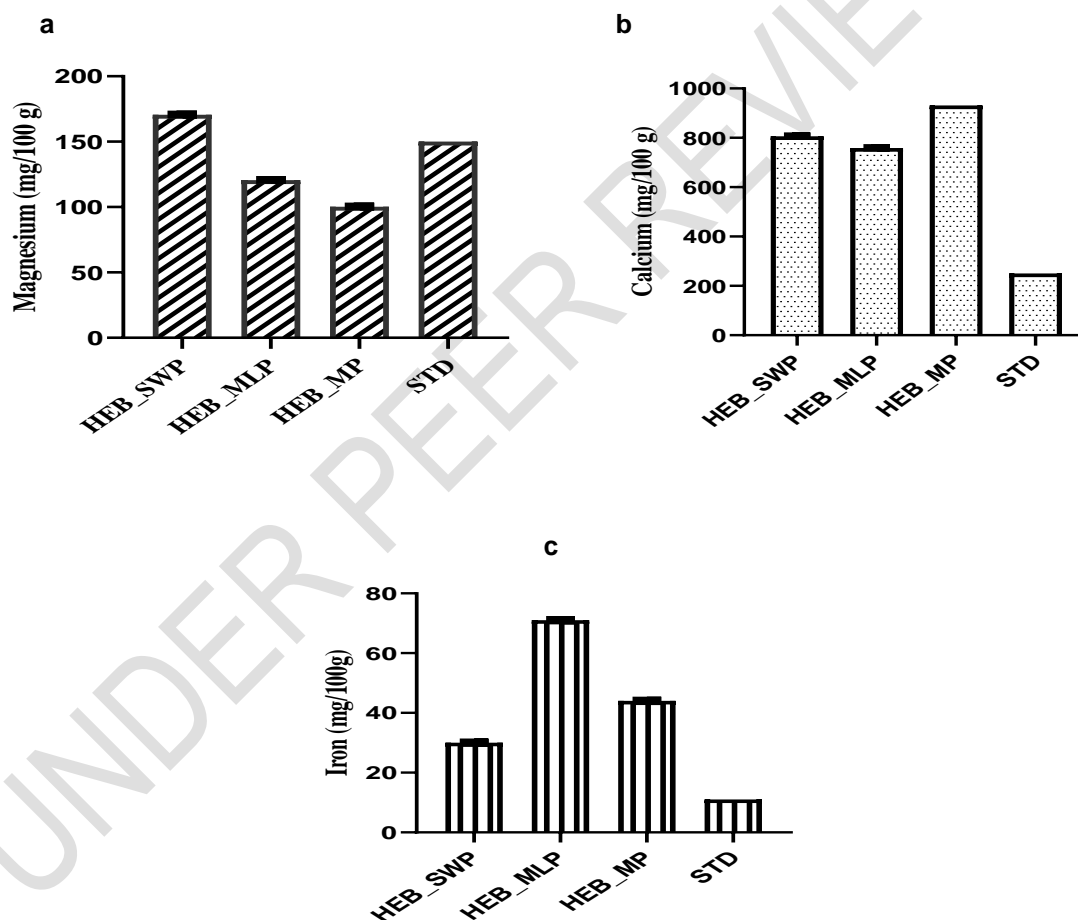
According to Akhtar and Isman [2], edible insects are excellent sources of vitamins and minerals. Figures 1 and 2 shows the vitamin and mineral composition of the rice biscuits relative to USAID [19] specifications. It was observed the HEB with migratory locust powder was significantly superior to other rice biscuits in terms of potassium, zinc and iron (Table 4 and Figure 1c). Iron, a vital nutritive element, is mostly absorbed through consumption of meat rather than plant-based meals. Iron deficiency may be common in populations who consume little or no meat or fish, as well as in developing and underdeveloped countries, particularly among children and women of reproductive age. This is due to culture restrictions, little or no purchasing power for conventional proteins sources such as beef, fish, chicken, egg amongst others. According to Muller and Krawinkel [25], over two billion persons suffer from iron deficiency anemia globally. Insects and insect food products which are high in iron may be a beneficial source of nourishment for those lacking this mineral. Ooninx *et al.* [26] reported that *Locusta migratoria* have iron content (8 – 20 mg and 31 – 77 mg/100g of dry matter, respectively) higher than beef (6 mg/100 g of dry weight). The calcium and iron contents of the high energy rice biscuits were above the minimum standard requirements. Although, milk enriched HEB had higher calcium content than its counterparts. Milk is known to be a good source of calcium [27].Comparatively, magnesium and phosphorus as shown in Figure 1a and Table 4, were significantly higher in mulberry silkworm pupae enriched rice biscuit. The insect-enriched rice biscuits contained above five times the minimum standard level (250  $\mu$ g/100 g) of pro-vitamin A. This can be attributed to the presence of the orange-fleshed sweet potato. Hence, these rice biscuits can serve as functional products to mitigate against incidences of hidden hunger (micronutrient deficiencies) in vulnerable populace.

**Table 4. Mineral composition of the enriched rice biscuits**

Sample (mg/100 g)	Phosphorus	Potassium	Zinc
HEB_SWP	66.70 ± 0.12 <sup>a</sup>	41.69 ± 0.19 <sup>b</sup>	6.00 ± 0.02 <sup>c</sup>
HEB_MLP	51.80 ± 0.05 <sup>c</sup>	52.55 ± 0.55 <sup>a</sup>	11.00 ± 0.05 <sup>a</sup>
HEB_MP	62.00 ± 0.02 <sup>b</sup>	30.00 ± 0.01 <sup>c</sup>	7.60 ± 0.02 <sup>b</sup>

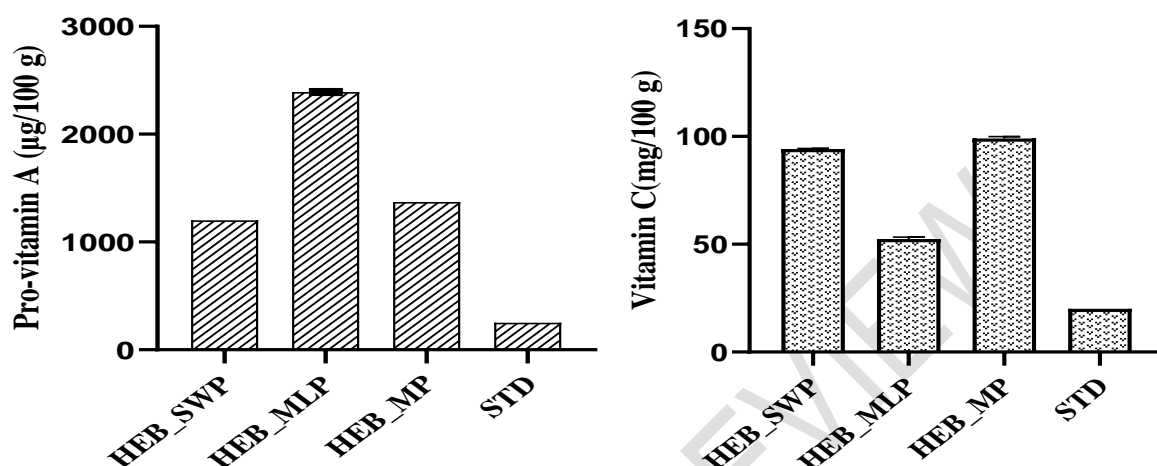
All values are mean ±SD of three replicates. Values with different alphabet in the same column are significantly different ( $p = 0.05$ )

HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder



**Figure 1. Magnesium (a), iron (b) and calcium (c) contents of the high energy rice biscuits compared with the minimum USAID standard for energy biscuits**

HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder; STD: USAID [19] standards for high energy biscuits



**Figure 2.** Pro-vitamin A and Vitamin C contents of the high energy rice biscuits compared with the minimum USAID standard for energy biscuits  
**HEB\_SWP:** High energy rice biscuit with silkworm pupae powder; **HEB\_MLP:** High energy rice biscuit with migratory locust powder; **HEB\_MP:** High energy rice biscuit with milk powder; **STD:** USAID [19] standards for high energy biscuits

### 3.4 Microbial qualities of high energy rice biscuits

The risks associated with insect-based products demands a detailed microbiological investigation of insect products intended for human consumption. From the study, all the samples were free of coliforms, *Escherichia coli* and *Salmonella* spp (Table 5). Mancini *et al.* [28] and Grabowski & Klein [29] also reported the absence of *E.coli* and *Salmonella*spp in blanched mealworm (*Tenebrio molitor*) and deep-fried spiced *Locusta migratoria*, respectively. The presence of aerobic mesophilic bacteria, *Staphylococcus aureus* and yeasts and mouldswere detected in all the high energy rice biscuits and *Bacillus cereus* in milk and migratory locust enriched biscuits, respectively. However, the products were within USAID 2016 standard microbial safety requirements for high energy biscuits.

**Table 5.** Microbial properties of the high energy rice biscuits

Agar	Microbes	HEB_SWP (cfu/g)	HEB_MLP (cfu/g)	HEB_MP (cfu/g)	STD* (cfu/g)
NA	AMB	$1.0 \times 10^{-2}$	$6.5 \times 10^{-2}$	$1.5 \times 10^{-2}$	<10,000
NA	<i>Bacillus cereus</i>	nil	$0.5 \times 10^{-2}$	$5.5 \times 10^{-2}$	<10
MCA	Coliform	nil	nil	nil	<10
EMB	<i>Escherichia coli</i>	nil	nil	nil	Absent in 1g
SSA	Salmonella	nil	nil	nil	Absent in 25g
MSA	<i>S. aureus</i>	$4.5 \times 10^{-2}$	$4.5 \times 10^{-2}$	$5.0 \times 10^{-2}$	<10
PDA	Yeasts & moulds	$3.0 \times 10^{-2}$	$1.0 \times 10^{-2}$	$5.5 \times 10^{-2}$	<100

All values are mean  $\pm$ SD of three replicates. Values with different alphabet in the same row are significantly different ( $p = 0.05$ )

NA: Nutrient agar; MCA: MacConkey agar; EMB: Eosin methylene blue; SSA: Salmonella-Shigella agar; MSA: Mannitol salt agar; PDA: Potato dextrose agar, AMB: Aerobic Mesophilic Bacteria; HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder; \*USAID [19] standards for high energy biscuit, S: *Staphylococcus*

### 3.5 Sensory attributes of high energy biscuit

Organoleptic aspects play a pivotal role in the acceptance of a novel product inspite of its nutritional quality. No statistical difference ( $p > 0.05$ ) was observed for the colour, crispness and appearance of the energy biscuits (Table 6). Migratory locust-enriched HEB was ranked higher in terms of taste, flavour and overall acceptability. This is contrary to high energy maize biscuits and cricket biscuits reported by Akande *et al.* [6] and Homann *et al.* [4], respectively. In these two cases, the milk biscuits were preferred above the insect-enriched biscuits. The migratory locust powder gave the biscuit a darker shade which was preferred by the panelists. All the biscuits were moderately liked by the panelists.

**Table 6. Sensory attributes of the enriched biscuits**

Sample	Colour	Taste	Appearance	Flavour	Crispness	OA
HEB_SWP	6.50 $\pm$ 0.26 <sup>a</sup>	5.85 $\pm$ 0.41 <sup>b</sup>	6.60 $\pm$ 0.20 <sup>a</sup>	5.70 $\pm$ 0.34 <sup>b</sup>	7.30 $\pm$ 0.16 <sup>a</sup>	6.35 $\pm$ 0.26 <sup>b</sup>
HEB_MLP	6.65 $\pm$ 0.29 <sup>a</sup>	7.20 $\pm$ 0.23 <sup>a</sup>	6.80 $\pm$ 0.23 <sup>a</sup>	7.15 $\pm$ 0.30 <sup>a</sup>	6.75 $\pm$ 0.19 <sup>a</sup>	7.30 $\pm$ 0.25 <sup>a</sup>
HEB_MP	6.65 $\pm$ 0.23 <sup>a</sup>	5.70 $\pm$ 0.46 <sup>b</sup>	6.95 $\pm$ 0.28 <sup>a</sup>	5.65 $\pm$ 0.45 <sup>b</sup>	7.15 $\pm$ 0.22 <sup>a</sup>	6.75 $\pm$ 0.38 <sup>ab</sup>

All values are mean  $\pm$ SD of three replicates. Values with different alphabet in the same column are significantly different ( $p = 0.05$ )

HEB\_SWP: High energy rice biscuit with silkworm pupae powder; HEB\_MLP: High energy rice biscuit with migratory locust powder; HEB\_MP: High energy rice biscuit with milk powder; OA: Overall acceptability

## 4. CONCLUSION

The study demonstrated the potential of development of high energy biscuits from rice and other indigenous ingredients such as OFSP flour, moringa leaf powder and edible insect powders. The nutritional profile of the insect biscuits also emphasized the fact that migratory locust and mulberry silkworm pupae powders can compete with conventional proteins such as skimmed milk in the development of therapeutic products such as high energy biscuits. Silkworm pupa biscuit exhibited higher protein, fat, crude fibre and ash contents while migratory locust biscuit had lower moisture, higher carbohydrate and energy contents coupled with better sensory acceptability. Aside from magnesium in which the migratory locust rice-HEB was marginally below the minimum standard, the insect-enriched biscuits exceeded the minimum requirements for minerals, pro-vitamin A and vitamin C. Additionally, the insect-enriched biscuits had good microbial status as it was free from Coliforms, *Escherichia coli*, *Salmonella* and had permissible levels of other microorganisms. Based on these findings, insect-enriched high energy biscuits could serve a sustainable alternative for alleviation of protein energy malnutrition and micronutrient deficiencies.

## REFERENCES

1. Rumpold BA, Schlüter OK. Potential and challenges of insects as an innovative source for food and feed production. *IFSET*. 2013;17:1-1.
2. Akhtar Y, Isman MB. Insects as an alternative protein source. In *Proteins in food processing 2018*;1 (pp. 263-288). Woodhead Publishing.
3. DeFoliart GR. Insects as food: why the western attitude is important. *Annu.Rev. Entomol.* 1999; 44(1):21-50.
4. Homann AM, Ayieko MA, Konyole SO, Roos N. Acceptability of biscuits containing 10% cricket (*Acheta domesticus*) compared to milk biscuits among 5-10-year-old Kenyan schoolchildren. *JIFF*. 2017;3(2):95-103.
5. Awobusuyi TD, Pillay K, Siwela M. Consumer acceptance of biscuits supplemented with a sorghum–insect meal. *Nutrients*. 2020;12(4):895.
6. Akande AO, Jolayemi OS, Adelugba VA, Akande ST. Silkworm pupae (*Bombyx mori*) and locusts as alternative protein sources for high-energy biscuits. *J. Asia. Pac. Entomol.* 2020;23(1):234-41.
7. Akande OA, Falade OO, Badejo AA, Adekoya I. Assessment of Mulberry Silkworm Pupae and African Palm Weevil larvae as alternative protein sources in snack fillings. *Heliyon*. 2020;6(4):e03754
8. Parker ME, Zobrist S, Lutterodt HE, Asiedu CR, Donahue C, Edick C, Mansen K, Pelto G, Milani P, Soor S, Laar A. Evaluating the nutritional content of an insect-fortified food for the child complementary diet in Ghana. *BMC Nutr.* 2020;1-11.
9. Akande OA, Oluwamukomi M, Osundahunsi OF, Ijarotimi OS, Mukisa IM. Evaluating the potential for utilising migratory locust powder (*Locusta migratoria*) as an alternative protein source in peanut-based ready-to-use therapeutic foods. *FSTI*. 2023;(3):204-16.
10. Akande OA., Oluwamukomi M, Osundahunsi OF, Ijarotimi OS, Ologbon AO. Evaluation of the Microbial Quality and Consumer Acceptability Peanut-Based Ready-To-Use Therapeutic Foods (RUTF) enriched with Migratory Locust (*Locusta Migratoria*) Powder. *ATA*. 2023;28(1):318-27.
11. Ivanišová E, Rajnoha M, Harangozo L, Kunecová D, Čech M, Gabriny L, Gálik B, Banach JK, Kowalczewski PŁ, Pietrzak-Fiećko R. Physicochemical, Nutritional, Antioxidant, and Sensory Properties of Crackers Supplemented with Edible Insects. *Appl. Sci.* 2023;13(21):11911.
12. Zazueta-Morales JJ, Martínez-Bustos F, Jacobo-Valenzuela N, Ordorica-Falomir C, Paredes-López O. Effect of the addition of calcium hydroxide on some characteristics of extruded products from blue maize (*Zea mays L*) using response surface methodology. *J. Sci. Food Agric.* 2001;81(14):1379-86.
13. Association of Analytical Chemists (AOAC). *Official Methods of Analysis*. 19th Edition. *Association of Official Analytical Chemists*, Washington, DC, 2012

14. Association of Analytical Chemists (AOAC). Official methods of analysis. In: Horowitz, W. (Ed.), *Official Methods of Analysis*, 18th ed. AOAC Publisher, Gaithersburg, USA, 2005
15. Jacob AG, Etong DI, Tijjani A. Proximate, mineral and anti-nutritional compositions of melon (*Citrullus lanatus*) seeds. *Br. J. Res.* 2015;2(5):142-51.
16. De Carvalho LM, Gomes PB, de Oliveira Godoy RL, Pacheco S, do Monte PH, de Carvalho JL, Nutti MR, Neves AC, Vieira AC, Ramos SR. Total carotenoid content,  $\alpha$ -carotene and  $\beta$ -carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. *Food Res. Int.* 2012;47(2):337-40.
17. Kapur A, Hasković A, Čopra-Janićijević A, Klepo L, Topčagić A, Tahirović I, Sofić E. Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. *Glas. hem. tehnol. Bosne Herceg.* 2012;38(4):39-42.
18. Harrigan WF, McCance ME. *Laboratory methods in food and dairy microbiology*. Academic Press, London, 1990.
19. United States Agency International Development (USAID). Multi-sectoral nutrition strategy 2014 – 2025: Nutrition in emergencies. Technical Guidance Brief. 2016;1–8.
20. Pauter P, Róžańska M, Wiza P, Dworzak S, Grobelna N, Sarbak P, Kowalczewski PŁ. Effects of the replacement of wheat flour with cricket powder on the characteristics of muffins. *Acta Sci. Pol. Technol. Aliment.* 2018;17(3):227-33.
21. Ouma FO. *Nutritional Composition, Microbiological Assessment, Shelf-Life and Sensory Properties of Wheat Muffins Enriched With African Emperor Moth (Gonimbrasia Zambesina, W) Caterpillar (S) Flour*. 2023 (Doctoral dissertation, JOOUST).
22. Sriprablom J, Kitthawee S, Suphantharika M. Functional and physicochemical properties of cookies enriched with edible insect (*Tenebrio molitor* and *Zophobas atratus*) powders. *J. Food Meas. Charact.* 2022;16(3):2181-90.
23. Dobermann D, Swift JA, Field LM. Opportunities and hurdles of edible insects for food and feed. *Nutr. Bull.* 2017;42(4):293-308.
24. Drakos A, Andrioti-Petropoulou L, Evageliou V, Mandala I. Physical and textural properties of biscuits containing jet milled rye and barley flour. *JFST.* 2019;56:367-75.
25. Müller O, Krawinkel M. Malnutrition and health in developing countries. *Cmaj.* 2005;173(3): 279-286.
26. Oonincx DG, Van der Poel AF. Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). *Zoo Biol.* 2011;30(1):9-16.
27. Ratajczak AE, Zawada A, Rychter AM, Dobrowolska A, Kreła-Kaźmierczak I. Milk and dairy products: good or bad for human bone? practical dietary recommendations for the prevention and management of osteoporosis. *Nutrients.* 2021;13(4):1329.
28. Mancini S, Fratini F, Tuccinardi T, Turchi B, Nuvoloni R, Paci G. Effects of different blanching treatments on microbiological profile and quality of the mealworm (*Tenebrio molitor*). *JIFF.* 2019;5(3):225-34.
29. Grabowski NT, Klein G. Microbiology of processed edible insect products—results of a preliminary survey. *Int. J. Food Microbiol.* 2017;243:103-7.

UNDER PEER REVIEW